

THE STUDY OF ALKALOID EXTRACTS EFFECT OF NERIUM OLEANDER AND APIUM GRAVEOLENS IN THE BODY WEIGHT AND BLOOD PARAMETERS IN LABORATORY MICE FEMALES *MUS MUSCULUS L.*

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ABSTRACT

The results of study were showed significant decrease in body weight of mice females groups treated with alkaloid extracts of Nerium oleander (Oleander) and Apium graveolens (Celery) compared with control group. As showed significant decrease in Red Blood Corpuscles account (RBCs) and Packed Cell Volume (PCV) and Hemoglobin (Hb) exception of the second group in the case (RBCs) and significant increase in (WBC) and Red Blood Distribution Width (RDW). As well as the results were showed no significant differences in Mean Corpuscular Volume (MCV) and significant decrease in Mean Corpuscular Hemoglobin (MCH) and significant differences in Mean Corpuscular Hemoglobin Concentration (MCHC) in mice Females groups treated with alkaloid extracts of Nerium oleander (Oleander) and Apium graveolens (Celery) compared with control, As the results were showed significant increase in Platelet account (PLT) and Plateletcrit (PCT) and significant decrease in Mean Platelet Volume (MPV) in mice Females groups treated with alkaloid extracts of Nerium oleander (Oleander) and Apium graveolens (Celery) compared with control group. The current study aimed to study the effect of alkaloid extracts of Nerium oleander (Oleander) and Apium graveolens (Celery) in the Body weight and Blood Parameters in mice females.

KEYWORDS: Alkaloid, Apium graveolens, Nerium oleander, Blood Parameters

Original Article

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INTRODUCTION

Alkaloids are secondary metabolites of plants (Hossain *et al.*, 2011) and represent a very diverse group of medically significant compounds, They were originally defined as pharmacologically active, nitrogen-containing basic compounds of plant origin (Lopes *et al.*, 2009). They have also been isolated from numerous animal sources (Buchanan *et al.*, 2002). Alkaloids are usually organic bases and form salts with acids and when soluble gives alkaline solutions (Stary, 1998). Many alkaloids, though poisonous, have physiological effect that renders them valuable medicine against various diseases including malaria, diabetics, cancer, cardiac dysfunction (Kumar & Sachin, 2013.), and lead a major impact throughout history on the economic, medical, political and social affairs of humans (Shamsa *et al.*, 2008). Some alkaloids effect on blood parameters as lead to reduce concentration of hemoglobin and Red Blood Corpuscles in rabbits fed on potatoes infected with Greenness (Hellenas, *et al.*, 2992).

MATERIALS AND METHODS

Experimental Animals

The animals used in this study were 30 Female Albino Mice type *Mus musculus*, Balb / c strain weighing between 21-23 g. bred in the animal house of College Education for pure sciences, University of Thi-Qar, They

were kept in mice cages and fed commercial feeds and allowed free access to clean fresh water in bottles *ad libitum*.

Alkaloids Extraction

Alkaloid extracts were attended using the method (Harborne, 1984).

Alkaloids Test

Reagent	Nerium Oleander	Apium Graveolens
Dragendroffs	+	+
Wagners	+	+
Marqus	+	+

Experimental Designs

The experimental mice were divided into five groups (6 animals in each group):

- The First Group (control): Injected with (0.1) ml / body weight physiological solution.
- The Second group: Injected with 0.1 alkaloid extract of *Nerium oleander* leaves (20 mg/Kg).
- The Third group: Injected with 0.1 alkaloid extract of *Nerium oleander* leaves (40 mg / Kg).
- The Fourth group: Injected with 0.1 alkaloid extract of *Apium graveolens* leaves (20 mg/Kg).
- The Fifth group: Injected with 0.1 alkaloid extract of *Apium graveolens* leaves (40 mg/Kg).

All individuals in these groups were injection via intraperitoneal membrane (IP) per day for a period of 30 days.

The Collection of Blood Samples

Blood samples were collected via heart puncture technique, after the anesthesia of animals with chloroform and taking blood directly from the heart using of the medical syringes with a capacity (1 ml) and preserved in tubes container on the anti-clotting substance EDTA to conduct blood tests.

Determination the difference of Body Weight Average for Laboratory Mice Females

The control group and the total of animals were treated with alkaloid extracts of *Nerium oleander* and *Apium graveolens* were weighted four times) in the first day of the experiment and after 10 days and 20 days and after the last day of the experiment using a sensitive balance and being account animals weight difference.

Determination of Some Blood Parameters of Laboratory Mice Females

This side included calculation of blood parameters using Coulter horiba apparatus include:

- Calculation of Red Blood Corpuscles counts (RBC) and Packed Cell Volume (PCV) and Hemoglobin (Hb) and Red Blood Cell Distribution Width (RDW) and White Blood Cells (WBC).
- Calculation of Mean Corpuscular Volume (MCV) and Mean Corpuscular Hemoglobin (MCH) and Mean Corpuscular Hemoglobin Concentration (MCHC).
- Calculation of Platelet (PLT) and Mean Platelet Volume (MPV) and Plateletcrit (PCT).

Statistical Analysis

Data were statistically analyzed using (SPSS) statistical software version (14) by one way ANOVA test using Least a Significant Difference (L.S.D) at level probability ($P \leq 0.05$).

RESULTS

The results in table 1 and showed a significant decrease ($p < 0.05$) in body weight after the first ten days of experience in females treated with alkaloid extracts of *Nerium oleander* and *Apium graveolens* compared with control group. And not significant decrease in the third group, compared with the second group and in the fourth group compared with the fifth group at ($p < 0.05$). Also the results also showed a significant decrease ($p < 0.05$) in body weight after 20 days of experience in females treated with alkaloid extracts when compared with the control group, and decrease was not significant in the second group compared with third group in fourth group compared with fifth group at ($p < 0.05$). As for after 30 days from the experience results showed significant decrease ($p < 0.05$) in body weight in females treated with alkaloid extracts when compared with the control group, and a significant decrease in the third group compared with the second group and not significant decrease in fifth group compared with the fourth group.

The results in table 2 and explained a significant decrease ($p \leq 0.05$) in RBCs in females treated with alkaloid extracts except the second group when compared with the control group, and decrease was not significant in the third group compared with the the second group and in fifth group compared with the fourth group at ($p \leq 0.05$). The results of the study significant decrease in PCV at ($p \leq 0.05$) in females treated with alkaloid extracts when compared with the control group, and a significant decrease in the third group compared with the the second group, and not significant in the fifth group compared with the fourth group at ($p \leq 0.05$).

Results of the study also showed a significant decrease in Hb at ($p \leq 0.05$) in females treated with alkaloid extracts when compared with the control group, and a significant decrease in the third group compared with the second group, and not significant in fifth group compared with the fourth group.

As the results of the study showed increase significant in RDW at ($p \leq 0.05$) in females treated with alkaloid extracts when compared with the control group and also showed significant increase in the second group compared to the third group and in the fourth group compared to the fifth group. The results of the study also revealed significant increase in WBC at ($p \leq 0.05$) in females treated with alkaloid extracts when compared with the control group and significant increase in the third group compared with the second group and in the fifth group, compared with the fourth group.

Results show in Table 3 and no significant differences in MCV at ($p \leq 0.05$) in females treated with alkaloid extracts when compared with the control group. The results also showed a significant decrease in MCH in females treated with alkaloid extracts when compared with the control group at ($p \leq 0.05$), and a significant decrease in the third group compared with the second group and in the fifth group compared with the fourth group and showed significant differences in the MCHC at ($p \leq 0.05$) in females treated with alkaloid extracts when compared with the control group and indicated significant increase in the second group compared with the third group and in the fourth group compared with fifth group.

The results showed in Table 4 and significant increase in PLT at ($p \leq 0.05$) in females treated with alkaloid extracts when compared with the control group and also showed significant increase in the third group compared with the second group and in the fifth group, compared with the fourth group. and also noted the a significant decrease in the MPV in females treated with alkaloid extracts when compared with the control group at ($p \leq 0.05$), and a significant decrease in the

second group compared with the third group and the decrease not significant in the fourth group compared with fifth group. Also the results displayed significant increase in Pct in females treated with alkaloid extracts exception of (the fourth group), when compared with the control group at ($p \leq 0.05$) and significant increase in the third group compared with the second group and in the fifth group, compared with the fourth group at ($p \leq 0.05$).

Table 1: Showed Effect Alkaloid Extracts of *Nerium Oleander* and *Apium Graveolens* in Body Weight for Laboratory Mice Females) Mean \pm Standard Error) (N =6)

Groups \ Parameters	First Day (gm)	After 10 Days (gm)	After 20 Days (gm)	After 30 Days (gm)
The first group (control) 0.1 ml /body weight from physiological solution	24.17 a ± 0.33	24.42 a ± 0.42	23.75 a ± 0.34	25.42 a ± 0.31
The second group (alkaloid extract of <i>Nerium oleander</i> 20 mg/Kg)	23.75 a ± 0.21	22.40 b ± 0.31	21.50 bd ± 0.43	22.30 b ± 0.29
The third group (alkaloid extract of <i>Nerium oleander</i> 40 mg/Kg)	24.33 a ± 0.25	22.08 b ± 0.41	22.58 b ± 0.51	20.58 c ± 0.52
The fourth group (alkaloid extract of <i>Apium graveolens</i> 20 mg/Kg)	23.67 a ± 0.17	21.75 bc ± 0.38	20.58 cd ± 0.30	21.67 bd ± 0.17
The fifth group (alkaloid extract of <i>Apium graveolens</i> 40 mg/Kg)	24.17 a ± 0.25	22.00 bc ± 0.39	21.66 bc ± 0.34	20.83 cde ± 0.21
L.S.D	0.71	1.13	1.15	0.94

*The different characters refers to the significant variations

Table 2: Showed Effect Alkaloid Extracts of *Nerium Oleander* and *Apium Graveolens* in Blood Parameters for Laboratory Mice Females) Mean \pm Standard Error) (N =6)

Groups \ Parameters	RBCs ($\times 10^6/\text{mm}^3$)	PCV (%)	Hb (g/dL)	RDW (%)	WBC ($\times 10^3/\text{mm}^3$)
The first group (control) 0.1 ml/ body weight from physiological solution	8.40 a ± 0.17	41.67 a ± 0.61	12.13 a ± 0.26	12.43 a ± 0.36	5.50 a ± 0.15
The second group (alkaloid extract of <i>Nerium oleander</i> 20 mg/Kg)	7.58 abcd ± 0.27	35.12 b ± 0.60	10.07 b ± 0.11	27.43 b ± 0.51	6.97 b ± 0.31
The third group (alkaloid extract of <i>Nerium oleander</i> 40 mg/Kg)	6.85 b ± 0.65	31.17 c ± 1.07	8.38 c ± 0.35	16.77 c ± 0.51	11.53 c ± 0.52
The fourth group (alkaloid extract of <i>Apium graveolens</i> 20 mg/Kg)	7.03 bc ± 0.45	33.53 bd ± 0.38	9.40 bc ± 0.07	29.08 bd ± 1.64	10.35 d ± 0.35
The fifth group (alkaloid extract of <i>Apium graveolens</i> 40 mg/Kg)	6.95 bc ± 0.40	32.85 cde ± 0.78	8.52 cd ± 0.72	16.27 ce ± 0.35	15.23 e ± 0.55
L.S.D	1.24	2.12	1.12	2.41	1.17

*The different characters refers to the significant variations

Table 3: Showed Effect Alkaloid Extracts of *Nerium Oleander* and *Apium Graveolens* in Constants of RBC for Laboratory Mice Females) Mean \pm Standard Error) (N =6)

Parameters Groups	MCV (fL)	MCH (pg)	MCHC (g/dL)
The first group (control) 0.1 ml/ body weight from physiological solution	46.83 a \pm 0.48	14.03 a \pm 0.15	27.68 a \pm 0.43
The second group (alkaloid extract of <i>Nerium oleander</i> 20 mg/Kg)	46.37 a \pm 0.74	13.22 b \pm 0.15	29.34 b \pm 0.63
The third group (alkaloid extract of <i>Nerium oleander</i> 40 mg/Kg)	45.50 a \pm 0.76	12.28 c \pm 0.35	27.00 a \pm 0.46
The fourth group (alkaloid extract of <i>Apium graveolens</i> 20 mg/Kg)	47.38 a \pm 1.34	13.20 b \pm 0.32	29.25 b \pm 0.69
The fifth group (alkaloid extract of <i>Apium graveolens</i> 40 mg/Kg)	47.33 a \pm 0.99	12.27 c \pm 0.33	25.95 c \pm 0.36
L.S.D	2.65	0.80	1.56

*The different characters refers to the significant variations

Table 4: Showed Effect Alkaloid Extracts of *Nerium Oleander* and *Apium Graveolens* in Platelets for Laboratory Mice Females) Mean \pm Standard Error) (N =6)

Parameters Groups	PLT ($\times 10^3/\text{mm}^3$)	MPV (fL)	PCT($\times 10^{-2}$) (%)
The first group (control) 0.1 ml/ body weight from physiological solution	631.67 a \pm 20.19	10.12 a \pm 0.36	22 a \pm 0.04
The second group (alkaloid extract of <i>Nerium oleander</i> 20 mg/Kg)	1080.67 b \pm 6.49	6.13 b \pm 0.33	58 b \pm 0.04
The third group (alkaloid extract of <i>Nerium oleander</i> 40 mg/Kg)	1269.50 c \pm 5.37	7.30 c \pm 0.32	91 c \pm 0.04
The fourth group (alkaloid extract of <i>Apium graveolens</i> 20 mg/Kg)	999.67 d \pm 0.71	6.20 b \pm 0.29	33 ab \pm 0.03
The fifth group (alkaloid extract of <i>Apium graveolens</i> 40 mg/Kg)	1533.17 e \pm 10.44	6.65 bc \pm 0.48	105 ce \pm 0.20
L.S.D	30.60	1.05	0.28

*The different characters refers to the significant variations

DISCUSSIONS

Effect of Alkaloid Extracts of *Nerium Oleander* and *Apium Graveolens* in Body Weight for Laboratory Mice Females

The Result of the study showed a significant decrease in body weight after 10 days and 20 days and the last day of the injection in all groups treated with alkaloid extracts of *Nerium oleander* and *Apium graveolens* compared with the control group, the reason in the decrease of body weight may be is the loss of the animals treated with alkaloid extracts of appetite and lack of desire to eat food and then reduced weight, if pointed (Hamden *et al.*, 2013) that the alkaloids reduces the animal's appetite and its desire to eat their diet as a result of linked enzymes digestive and then lead to a loss of appetite and the occurrence of nutritional deficiencies which resulting in weight loss. Or may be caused by the lack of weight to the effect of alkaloids extracts in the central nervous system and cause convulsions and temporary paralysis and ataxia of the animal and being idle and then not being able to eat food normally has been clearly evident in animal control when compared with the control group animals, if pointed (Kritchevsky, 1991; Effraim *et al.*, 2000) to the lack of animal desire

in food intake due to some alkaloids affect the central nervous system of the animal and indirectly on the satiety centers in the brain and the loss of the animal to feel hungry and not to eat food that results in a decrease in body weight.

The results of the study agreed with the results of the study (Boghossian, 2011) which showed a decrease in body weight in mice treated with Morphine alkaloid compared with the control group, and disagreed with the results of the study (Abdelouahab *et al.*, 2011), which reached an increase in females rats treated with alkaloid extracts of *Datura stramonium* and a with concentration of 100 mg / kg for five days of treatment.

Effect of Alkaloid Extracts of *Nerium Oleander* and *Apium Graveolens* in the Blood Parameters for Laboratory Lice Females

The results the study showed significant decrease in RBC and Hb and PCV in the groups were treated with alkaloid extracts of *Nerium oleander* and *Apium graveolens* compared with the control group, this decrease may be due to one or more of the factors which involved in the blood reds cells manufacturing (Erythropoiesis process) or which the bone marrow (the production of RBC) or factor that catalyst to the process of production (the hormone erythropoietin), if pointed (Chatterjee and Deb, 1999) that alkaloid extracts from *Areca catechu* affect the bone marrow cells of mice when they are given by mouth, or injection into the peritoneal membrane as causing disorder in the formation of red blood cells and cause incompleteness.

May be caused by decrease in RBC is the Hemolysis and meant to crash or rupture of the membrane of RBC causing the liberation of hemoglobin Hb and internal components into the liquid, as explained (Takamura, 1998) that this mechanism occurs as a result of the disruption of ionic balance (osmosis ions) between the cell inside and abroad because of the effect of alkaloid extracts in the movement of fluids and ions through the cell membrane, as pointed (Bowman & Rand, 1980) that alkaloid extracts cause hemolysis in various cell types and in particular the RBC and cause lowering their counts in the bloodstream.

With regard to Hb and PCV decrease may be in the RBCs consequent decrease in Hb and PCV, of the fact that the relationship between RBC and this parameters is, extrusive, as explained (Akinloye *et al.*, 2014) that the relationship between Hb and with RBC extrusive increase upwards and decrease down, and this complies with what brought him the results of the current study in low Hb and PCV.

Results of the study agreed with the results of a study (Abdelouahab *et al.*, 2011) which showed that alkaloid extracts of seeds of *Datura stramonium* and a concentration of 100 mg / kg caused a decrease in RBC and Hb and PCV in female's laboratory mice after five days of treatment.

The results also showed a significant increase in WBC in the groups were treated with alkaloid extracts of *Nerium oleander* and *Apium graveolens* compared with the control group, the reason of increase those cells are due to that alkaloid extracts work as catalysts for cellular immunity, which is based on the production of antibodies, and humoral immunity which produced by lymphocytes, result from that increase in production and the high counts of WBC in the bloodstream, If noted (Nerurkail *et al.*, 2004). that the immune system is provoked with alkaloid extracts which entering the body through the injection and deal with it a foreign body (Antigen) and then happen increase the number of WBC, as indicated (Maizels & Yazdanbakhsh, 2003; Xingming *et al.*, 2009) that alkaloid extracts stimulate T-cell lymphocytes on production Interleukins IL 2 which raises the efficiency of the immune system and increase susceptibility to phagocytic cells to attack foreign bodies. The results of study agreed with the results of the study (Calapai *et al.*, 2009), which found that the nicotine

alkaloid works to increase WBC in rabbits, and disagreed with the results of study (Bouzidi *et al.*, 2011) which showed decrease the WBC in laboratory female rats treatment with alkaloid extract of *Datura stramonium* and a concentration of 100 mg / kg.

The results also showed a significant increase in RDW in the groups were treated with alkaloid extracts of *Nerium oleander* and *Apium graveolens* compared with the control group, The reason of rise in RDW may be due to anemia, which showed the results of the current study, through decrease RBCs and Hb and MCV, which may be result that animal not eating food because the loss of appetite and then occurs decrease in necessary iron equipping to configure the RBCs, if pointed (Viswanath *et al.*, 2001) that the incidence of Iron Deficiency Anemia causes a rise in RDW. The results disagreed with the results (Abdelouahab *et al.*, 2011) which showed that alkaloid extracts of *Datura stramonium* and a concentration of 100 mg / kg is working to reduce the RDW.

Effect of Alkaloid Extracts of Nerium Oleander and Apium Graveolens in the RBC Constants for Laboratory Mice Females

The results of study showed no significant differences in MCV in the groups were treated with alkaloid extracts of *Nerium oleander* and *Apium graveolens* compared with the control group, The results also showed a significant decrease in MCH in the treatment groups compared with the control, the decrease may be agreed with decrease of Hb and This is study reached by.

This decline may be due to decrease of iron in the body which essential in the formation of hemoglobin, If pointed (Al - Ganami, 2004) that iron deficiency leads to decrease the Hb in the RBCs, and this agreed with the results of study (Abdel *et al.*, 2006) which found that alkaloid extracts of Cinchona Tree reduced MCH, and this study differed with the results of a study (Sodipo *et al.*, 2013) which showed that alkaloid extracts from *Solanum macrocarpum* increases MCH.

Effect of Alkaloid Extracts of Nerium Oleander and Apium Graveolens in Platelets for Laboratory Mice Females

The results showed significant elevation in the counts of platelets and PCT in the groups were treated with alkaloid extracts of *Nerium oleander* and *Apium graveolens* compared with the control group, it may be the reason for the increase in the counts of platelets as a result of stimulating Megakaryocytes in the bone marrow to produce blood platelets necessary for the process of blood clotting and then increase their ratio in the bloodstream.

The results of the study agreed with the results of study (Al-Naqqash *et al.*, 2013) which found that alkaloid extracts of *Zingiber officinale* effective in reducing bleeding time and Clotting Time by increasing the counts of platelets, there is an inverse relationship between the time of bleeding and platelet count. As for the PCT, it would be Direct Proportion relationship with the counts of platelets, as pointed (Asif *et al.*, 2013) that whenever increase the counts of platelets increased PCT. also the results showed a significant decrease in MPV in the groups were treated with alkaloid extracts of *Nerium oleander* and *Apium graveolens* compared with the control group, may be caused by as a result of inflammation that get by the treatment of alkaloids extracts and their impact in the bone marrow or megakaryocyte cells, which negatively affects in MPV, If pointed (Gasparyan *et al.*, 2011) that in the case of inflammation increase the counts of platelets because of increasing the activity of inflammatory cytokines and accompanied an increase in crash of platelets and thus decreases in MPV, this results agreed with the results of study (Abdelouahab *et al.*, 2011) which showed that alkaloid extracts of seed *Datura stramonium* and a concentration of 100 mg / kg decrease MPV in laboratory mice female.

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